Rapid screening of cloned DNA fragments for specific mutations

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Site-specific mutagenesis is a powerful tool for altering DNA structure. After carrying out the manipulations required to introduce the nucleotide changes it is necessary to screen isolated plasmid clones to verify that the desired alteration was achieved. Frequently this is accomplished by engineering the mutation so that a new endonuclease recognition site is generated during the mutagenesis. Subsequent digestion by an enzyme that recognizes this site results in a restriction pattern which differs from that of the non-mutant. One limitation to this approach is that it can be difficult to design the appropriate mutation. Furthermore, the nucleotide substitutions that can be made are limited by the recognition sequences of suitable restriction endonucleases. Alternative approaches for identifying mutated, cloned DNA fragments include dot blot hybridization (1) and DNA sequencing of the mutated region. Both of these approaches have the disadvantage of being time consuming. Here we report a rapid screening assay for site-specific mutations using the polymerase chain reaction (PCR). This technique takes advantage of the fact that efficient DNA amplification by PCR requires two primers which have 3′ ends complementary to the target sequence.

The procedure described by Ho *et al.* (2) was utilized to introduce multiple nucleotide changes with a mutagenic oligonucleotide in an *erbB* oncogene cDNA at codons 477 and 478. DNA fragments were subcloned into a plasmid vector and transformed into *E. coli* DH5α. To determine which colonies contain plasmids encoding specific mutated *erbB* sequences, 1 nanogram of miniprep plasmid DNA was subjected to three PCR reactions. The reactions contained a 17-mer antisense primer complementary to the sequence located 500 base pairs upstream of the mutated codons. Each reaction also contained a 17-mer sense primer that has a 3′ end that is identical the desired codons encoding *erb*B amino acids 477 and 478. All reactions were carried out for 25 cycles in 1× Cetus buffer, 0.2 mM of each dNTP and 0.5 units of Taq DNA polmerase. The PCR thermal profile consisted of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 20 seconds. The data presented in figure 1 indicate that efficient amplification of plasmid DNA was achieved only when the sense primer has 3′ sequence that is complementary to the template DNA. As a result of these PCR reactions, it was possible to rapidly identify individual bacterial colonies containing the *erbB* oncogene with specific mutations at codons 477 and 478. The general utility of this technique should prove valuable in screening bacterial colonies for specific mutations in plasmid DNA.

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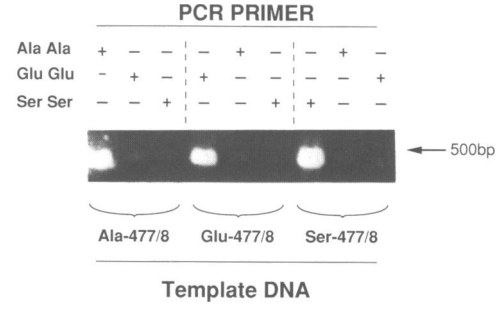

Figure 1. Screening of plasmid DNA mutated at *erbB* codons 477 and 478. Mutagenized *erbB* cDNA was used to transform *E. coli* DH5α. Plasmid DNA was isolated from the transformants and screened by PCR for the desired nucleic acid substitutions. All PCR reactions were carried out with a 17-mer antisense primer complementary to the sequence located 500 base pairs upstream of the mutated codons. As a second PCR primer, one of three 17-mer sense oligonucleotides was used. The structure of the sense oligonucleotides is: Ala Ala (5′ CCT GTT GGA TCT GCG GC 3′); Glu Glu (5′ CCT GTT GGA TCT TCT TC 3′) and Ser Ser (5′ CCT GTT GGA TCT GAG CT 3′).